

# Phosphorus Distribution in Soils Treated with Bioenergy Co-product Materials following Corn Growth

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## ABSTRACT

This research was conducted to investigate the impact of corn cob gasification biochar (CCGB), switchgrass pyrolysis biochar (SPB), turkey manure ash (TMA), and triple superphosphate fertilizer (TSP) on soil phosphorus (P) distribution in three agricultural soils from Minnesota, USA. Understanding how biochar can change soil P distribution is crucial to develop best management practices for recycling biochar products. Phosphorus sources were incorporated at rates of 0, 28, 56, and 84 mg  $P_2O_5$   $kg^{-1}$  to 1.5 kg of each soil in 2-L pots. Corn (*Zea mays* L.) plants were grown (2 plants  $pot^{-1}$ ) in treated soils for 56 d after emergence. After 56 d, plants were harvested and soil samples collected for sequential P fractionation ( $H_2O$ , 0.5 mol  $L^{-1}$   $NaHCO_3$ , 0.1 mol  $L^{-1}$   $NaOH$ , and 1.0 mol  $L^{-1}$   $HCl$ ) and enzymatic hydrolysis. The results of the sequential fractionation showed that CCGB and SPB were as effective as TSP and TMA at increasing total P extractable in water and  $HCl$ . In contrast, the increase in  $NaHCO_3$  and  $NaOH$  extractable total P was higher with TSP and TMA than with the CCGB and SPB. In most cases, the increase in inorganic P was similar between biochar and TSP, suggesting that biochar could supply equal amounts of plant available P as commercial fertilizer. The effects of biochar on enzymatically hydrolysable P were not consistent and varied by soil. In conclusion, the results of this study showed that biochar has potential to increase the available P pools in soils similar to commercial fertilizer.

## Core Ideas

- Biochar changes chemical distribution of P in soils.
- Biochar changes the distribution of hydrolysable organic P in soils.
- No two biochar materials are alike.

**P**YROLYSIS, GASIFICATION, and combustion are three processes being investigated and used to convert biomass into renewable energy and other products (Pagliari et al., 2010a; Spokas et al., 2012; Brown et al., 2015; Zheng et al., 2016). Differences in feedstock and incineration or combustion conditions (e.g., temperature, moisture content,  $O_2$  content) have an important effect on the physical and chemical properties of the resulting by-product, be it biochar (organic carbonaceous by-product of pyrolysis or gasification) or ash (inorganic mineral by-product of complete combustion) (Chia et al., 2015). The interactions between applied biochar and soil have been found to differ with biochar characteristics and soil properties (Pagliari et al., 2010b; Spokas et al., 2012).

In terms of nutrient retention, some biochars have a higher nutrient retention capacity than native soil organic matter (Lehmann, 2007a; Igalavithana et al., 2016). The nutrient retention capacity of biochar has been attributed to its high surface area, high cation exchange capacity, and high charge density (Liang et al., 2006; Igalavithana et al., 2016). The biochar surface chemistry is highly dependent on the production process, and the rates of heating and cooling have much more influence than the final heating temperature (Spokas and Novak, 2014). In addition to increasing soil cation exchange capacity, biochars have increased the retention of anionic phosphate anions ( $PO_4^{3-}$ ) in soils (Liang et al., 2006; Lehmann, 2007b; Steiner et al., 2007; Nelson et al., 2011). However, P retention by biochar-treated soil is poorly understood and varies among soil types and management practices. For example, some researchers reported that biochar increased P availability when supplemental inorganic P and nitrogen (N) were applied in tandem with biochar, as compared with soils that only received biochar (Steiner et al., 2007; Nelson et al., 2011). Such differences in P availability likely reflect differences in form and speciation of soil P that were driven by structural and chemical properties of biochars. It is still unclear whether biochar can contribute P to soils or if biochar acts on releasing P that is already present in the soil (Igalavithana et al., 2016).

Reported concentrations for P in biochars range from 0.01 to 6.0% (dry weight basis) depending on the original source material (Ippolito et al., 2015; Igalavithana et al., 2016). DeLuca et al.

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**Abbreviations:** CCGB, corn cob gasification biochar; SPB, switchgrass pyrolysis biochar; TMA, turkey manure ash; TSP, triple superphosphate fertilizer.

(2015) found that wood feedstock materials resulted in biochar with P concentrations of less than 0.1%, while manure feedstock resulted in biochar with P concentrations of 6.0%. Pyrolytic degradation of P-containing organic matter causes release of free orthophosphate groups that tend to precipitate with cations (e.g.,  $\text{Fe}^{3+}$ ,  $\text{Al}^{3+}$ ,  $\text{Ca}^{2+}$ ) found in the ash of pyrolyzed material or in soil to which biochar is applied (Pagliari et al., 2010a). The solubility and plant availability of precipitated metal-P complexes depends on their dissolution and precipitation behavior and the soil solution chemistry. In general, Ca-complexes tend to be more soluble than Fe- and Al-P precipitates (Havlin et al., 2005).

Most of the published research that investigated the interactions between biochar and soil primarily focused on nutrient (primarily P and N) availability (Liang et al., 2006; DeLuca et al., 2015). Fewer studies have investigated transformations among the various soil P pools (labile and stable and inorganic and organic) (Lehmann, 2007b). Sequential P fractionation, where the lability of soil P is characterized based on solubility in a series of extractants, has become a useful method to study soil P transformations and availability (Schmitt et al., 2017; Waldrip et al., 2015). A fairly standardized designation is separation of extractable-P into labile-P ( $\text{H}_2\text{O}$ -, resin-, and  $\text{NaHCO}_3$ -extractable P), moderately labile-P ( $\text{NaOH}$ -extractable P; assumed to be associated with amorphous Al and Fe oxides and OM), and stable-P ( $\text{HCl}$ -extractable P; assumed to be Ca-associated phosphates) (Hedley et al., 1982; Sui et al., 1999; Negassa and Leinweber, 2009). Non-extractable P is often designated as residual P. All extractable P fractions contain a given amount of P (total P [ $P_t$ ]) that can be divided into inorganic P ( $P_i$ ) and organic P ( $P_o$ ). The  $P_o$  can be further divided into several organic compounds that contain P. For soils, the most common studied  $P_o$  forms as assessed by hydrolysis with phosphatase enzymes with known specificities are enzymatically hydrolysable  $P_o$  ( $P_e$ ): monoester-like P, nucleotide-like P, phytate-like P; and non-hydrolysable  $P_o$  ( $P_{ne}$ ) (He and Honeycutt, 2001; He et al., 2003, 2004; Pagliari and Laboski, 2012). In cropping soils, the amount of labile  $P_i$  is also known as the soil P pool that is readily available for plant uptake, while the  $P_o$  is the pool that has potential to become available for plant uptake based on the potential for mineralization of any given soil and plant system. In aquatic ecosystems, labile or reactive  $P_i$  is the major component controlling eutrophication, while  $P_o$ , having not received as much focus as  $P_i$ , does not have a clear and well defined effect on how it impacts water quality.

Many methods can be employed to quantify each type of  $P_o$ , for example nuclear magnetic resonance, x-ray near-edge spectroscopy, or enzyme assays (Pagliari, 2014). Two samples are used for enzyme assays: (1) A solution containing specific enzymes is added and an incubation is performed, this will hydrolyze  $P_o$  into  $P_i$ , which is then determined by color; and (2) A background solution without enzymes is added and the sample undergoes the same incubation as the sample that received enzymes. By determining  $P_i$  in both samples, one can use the difference to determine the amount of each specific type of  $P_o$  present in each sample. In other words, if there is  $P_o$  in the sample there will be an increase in the  $P_i$  concentration of the sample that was incubated with the solution containing the known enzymes in comparison with the sample that did not receive the solution with enzyme.

Research has indicated that P is dynamic in soils, with constant transformations among the different pools, for example, inorganic to organic and labile to stable (Chen et al., 2002; Waldrip-Dail et al., 2009; He et al., 2011; Waldrip et al., 2011, 2012). Although recent studies investigated effects of biochars on soil test P, no work has evaluated their effects on the chemical distribution of soil P and susceptibility of soil  $P_o$  to phosphatase hydrolysis in the presence of growing crops. Some forms of  $P_o$  are easily converted into available P in soils; however, some forms of  $P_o$  have high affinity to sorb onto materials with high surface area, such as clay particles and potentially biochar particles, which would minimize their availability to hydrolysis and ultimately be less available for plant uptake (Pagliari and Laboski, 2013, 2014). The effects of biochar materials on soil chemical P fractions need to be clearly understood so that best management practices can be developed for adding biochar to cropped soils. In addition, because the  $P_o$  pool plays a significant role in both terrestrial and aquatic environments, it would be beneficial to start developing data that shows how biochar impacts this pool. Therefore, this study was designed to provide initial information regarding the effects of biochar on the different soil P pools of three soils with contrasting properties. The soils selected for this study represent soils that are used for intensive agricultural production and have potential to receive large amounts of biochar, once biochar application to soils becomes a common practice. The objectives of the current work were to evaluate the effects of biochars from two sources (corn cob gasification biochar [CCGB] and switchgrass pyrolysis biochar [SPB]), inorganic P fertilizer (triple superphosphate [TSP]), and ash from a turkey manure combustion facility (TMA), on short-term soil P chemical distribution in three soils from Minnesota after corn (*Zea mays* L.) growth for 56 d.

## MATERIALS AND METHODS

### Soil Collection and Experimental Setup

The three surface (0–15 cm) soil series were collected from fields used for conventional agriculture cropped with corn prior to collection: an excessively well-drained Hubbard loamy sand (pH 5.6) (central Minnesota; 45°41'10.40" N, 95°47'54.94" W); a poorly drained calcareous Canisteo clay loam (pH 6.1) (southern Minnesota; 45°23'32.24" N, 93°52'58.45" W); and a well-drained, Barnes loam (pH 8.1) (southwest Minnesota; 44°14'31.49" N, 95°18'02.36" W). All soils had soil test P levels (Bray-1 or Olsen) in the low category (Bray-1 <11 ppm; Olsen <8 ppm) (Rehm et al., 2006). The specific soil test P extraction methods used were Bray-1 when pH < 7.0 and Olsen when pH > 7.0. After collection, the soils were air-dried, ground to 2-mm, and stored in plastic containers until needed for treatment application. Subsamples of the air-dried ground soils were analyzed for total C, total N, ammonium ( $\text{NH}_4^+$ -N), nitrate ( $\text{NO}_3^-$ -N), pH, available potassium (K), and soil test P concentrations (Brown 1998). Total P concentrations were determined after nitric acid microwave digestion (Tandon et al., 1968). Selected soil properties are presented in Table 1.

Treatments consisted of a control (no P added) and three P rates (28, 56, and 84 mg  $\text{P}_2\text{O}_5$  kg<sup>-1</sup>) of each of the four treatments (TSP, TMA, CCGB, and SPB). Each treatment was

replicated four times. The TMA, a 60:40 blend of turkey manure and woody biomass, was obtained from a commercial power plant (Fibrominn LLC, Benson, MN), where incineration temperature was likely >850°C for optimal power production. The CCGB was a blend of corn cobs and woody biomass that underwent gasification (~850°C) in the presence of limited O<sub>2</sub> (equivalence ratio of 0.15 to 0.30 between O<sub>2</sub> admitted into the reactor and the moles of O<sub>2</sub> needed for complete combustion of the biomass). Switchgrass biochar was produced in a barrel batch pyrolysis reactor (provided courtesy of Dr. Harold Collins of USDA-ARS Grassland, Soil and Water Research Laboratory, Temple, TX). Each batch of SWB consisted of 32 kg of biomass. The reactor vessel was heated by an external wood source to 500°C (heating rate: 10°C min<sup>-1</sup>) and held (±20°) for 4 h at atmospheric pressure. An Omega Incorporated (Stanford, CT) XCIB-K-1-6 thermocouple was mounted inside the barrel and connected to a data logger (model X10, Campbell Scientific, Chesterland, OH) programmed to continuously record temperatures at 2-min intervals during pyrolysis. Upon batch completion the retort was allowed to air cool until biochar was removed.

Phosphorus sources were applied based on their total P concentration (Table 1) to achieve four P rates: 0, 28, 56, and 84 mg P<sub>2</sub>O<sub>5</sub> kg<sup>-1</sup> soil (dry matter basis). Table 1 provides a list of selected chemical properties for the TMA, CCGB, and SPB used in the study. The P sources were added to 1.5 kg of air-dried soil in 4-L plastic bags and thoroughly mixed. Treated soil mixtures were then placed in 2-L pots and packed to reach a bulk density of 1.2 g cm<sup>-3</sup>. Supplemental N and potassium (K) were provided by adding 112 mg N kg<sup>-1</sup> soil (as urea) and 56 mg K kg<sup>-1</sup> soil (as KCl) to assure that those nutrients were not limiting plant growth.

Four corn seeds were planted in each of the pots containing soil treatment. Seedlings were manually thinned to two plants per pot at 7 d after emergence. The growth portion of the experiment was conducted in a greenhouse where pots were organized according to a randomized complete block design on the greenhouse benches. A 14-h photoperiod was provided by fluorescent light. Daytime and nighttime temperatures were maintained between 21 and 27°C and 16 to 21°C, respectively. Water was provided daily to maintain soil at or near field capacity by weighing the pots and adding additional water to bring pot weight to initial values. At 56 d after emergence, plants were cut near the soil surface to determine nutrient uptake. In general, corn P uptake was low over the 56-d period (mean P uptake = 7.1 µg P pot<sup>-1</sup>) as compared with total extractable soil P. For example, the water-extractable P (mean H<sub>2</sub>O-P concentration = 6 mg P pot<sup>-1</sup>) was >1000-fold higher than plant P uptake. Therefore, plant P uptake can be disregarded as a source of variation in the soil P levels measured for the present study. Soil samples were collected, air-dried, ground (2-mm) and saved for chemical analysis. Plant roots were removed manually from the soil and shaken to remove any loosely attached soil. This rhizosphere soil was combined with bulk soil for each pot.

### Phosphorus Fractionation

The soil samples used in the sequential fractionation study included samples from each control soil (no P added) and samples from all treatments and replications after the greenhouse study. Phosphorus was extracted using the Hedley fractionation

Table 1. Chemical properties of turkey manure ash (TMA), switchgrass pyrolysis biochar (SPB), and corn-cob gasification biochar (CCGB) and selected soil properties for the three soils used in the study, Barnes, Canisteo, and Hubbard.

Chemical property	TMA	SPB	CCGB
Calcium Carbonate Equivalent (CCE) %	29.5	8.5	35.6
Effective Calcium Carbonate Equivalent (ECCE) %	22.7	6.6	35.4
pH (1:1 water)	12.0	9.4	11.4
Chloride (g kg <sup>-1</sup> )	25.1	ND†	3.5
Acid-digestible elements	g kg <sup>-1</sup>		
Calcium	162	11.6	130
Iron	4.3	0.40	3.5
Magnesium	17.3	12.0	6.5
Manganese	1.1	0.12	0.70
Phosphorus	46	4.5	1.4
Potassium	58.9	2.6	16.7
Sodium	13.0	0.6	2.2
Sulfur	15.2	1.3	ND
Zinc	0.87	0.10	0.14
	mg kg <sup>-1</sup>		
Arsenic	15.1	ND	8.1
Boron	98	ND	34
Cadmium	3.5	ND	1.4
Cobalt	3.9	ND	3.2
Copper	874	ND	46
Chromium	14.1	ND	7.2
Molybdenum	8.8	ND	9.2
Nickel	19.9	ND	10.5
Lead	52.3	ND	61.5
Selenium	10.8	ND	9.5
	Soil series		
Soil property	Barnes loam	Canisteo clay loam	Hubbard loamy sand
pH	8.1	6.1	5.6
Total C (g kg <sup>-1</sup> )	28.9	27.3	8.6
Total N (g kg <sup>-1</sup> )	22.9	24.7	8.5
NH <sup>+</sup> <sub>4</sub> -N (mg kg <sup>-1</sup> )	16.9	20.0	17.4
NO <sup>-</sup> <sub>3</sub> -N (mg kg <sup>-1</sup> )	10.4	4.8	3.0
Total P (mg kg <sup>-1</sup> )	575	482	526
Bray-1 P (mg kg <sup>-1</sup> )	—‡	8.1	8.5
Olsen P (mg kg <sup>-1</sup> )	2.9	—	—
Available K (mg kg <sup>-1</sup> )	183	96	34

† ND, below detection level.

‡ Not determined

method modified for livestock manure and manure-amended soil as described by He et al. (2006) and Waldrip-Dail et al. (2009). Soil (1.0 g) from each replicate pot was sequentially extracted with deionized H<sub>2</sub>O (25 mL) for 2 h at 22°C on an orbital shaker (250 rpm). Extracts were centrifuged at 23,700 g for 30 min at 4°C and supernatants were carefully decanted and passed through 0.45-µm nitrocellulose filters. Soil residues retained in the tubes were then sequentially extracted with 0.5 mol L<sup>-1</sup> NaHCO<sub>3</sub> (pH 8.5), 0.1 mol L<sup>-1</sup> NaOH, and 1.0 mol L<sup>-1</sup> HCl for 16 h each, with extraction conditions and supernatant collection as previously described. Deionized water was used to wash the samples between extractants to minimize contamination of subsequent fractions (He et al., 2006; Pagliari



and Laboski, 2012). The  $\text{NaHCO}_3$ ,  $\text{NaOH}$ , and  $\text{HCl}$  fractions were diluted and adjusted to pH 5.0 in 100-mmol  $\text{L}^{-1}$  sodium acetate buffer according to Waldrip-Dail et al. (2009). There were three major forms of P in each extract, total P ( $P_t$ ),  $P_i$ , and  $P_o$ , and they relate to each other as  $P_t = P_i + P_o$ . The  $P_o$  is further divided into two groups, hydrolysable P ( $P_e$ ) and non-hydrolysable P ( $P_{ne}$ ), such that  $P_o = P_e + P_{ne}$ . The  $P_e$  can then undergo enzymatically hydrolysis and specific  $P_o$  forms can be assigned. Total P in all four sequential fractions was determined by inductively coupled plasma–optical emission spectroscopy (ICP–OES) on a PerkinElmer 8x00 (PerkinElmer, Shelton, CT) according to do Nascimento et al. (2015). The concentration of  $P_i$  in all four sequential fractions was quantified on a Biotek Epoch spectrophotometer (Biotek, Winooski, VT) at a wavelength of 850 nm using a modification of the molybdenum blue method of Dick and Tabatabai (1977) as described by He and Honeycutt (2005). The  $P_o$  was determined by the difference  $P_t - P_i$ .

### Enzymatic Hydrolysis

The amount of  $P_e$  from each sequential fraction was quantified according to the method described by He and Honeycutt (2001). Briefly, an aliquot (0.3 mL) of each pH-adjusted and diluted P fraction (described above) was incubated with 0.2 mL of a sodium acetate (pH 5.0) enzyme solution. This final mixture contained 0.25 units  $\text{mL}^{-1}$  each of acid phosphomonoesterase (EC 3.1.3.2) type IV-S from potato and type I from wheat germ and 2 units  $\text{mL}^{-1}$  nuclease P1 from *Penicillium citrinum* (EC 3.1.30.1) (all enzymes purchased from Sigma-Aldrich, St. Louis, MO). Incubation using phosphomonoesterase from potato alone gives the amount of monoester-like P; incubation using phosphomonoesterase from potato and wheat germ in combination gives phytate-like P, while incubation with all three enzymes gives DNA-like P (He and Honeycutt, 2001). Therefore, in this study  $P_e = \text{monoester-like P} + \text{phytate-like P} + \text{DNA-like P}$ . Extracts were then incubated for 1 h at 37°C on a shaker incubator at 180 rpm. Assay controls were included where enzymes, substrate (P fraction), or both were omitted. All incubations were performed in triplicate. After incubation,  $P_i$  must be determined in all the samples so precise estimations can be calculated. For these samples,  $P_i$  was also determined by the molybdate blue method (He and Honeycutt, 2005). The concentration of  $P_i$  measured in the samples that undergo incubation without the addition of enzymes tends to be the same as that determined prior to the incubation (for samples being analyzed soon after the sequential fractionation as described in the previous section). However, in some cases small changes do happen in P concentrations during incubation, and those could hinder the ability to accurately detect each of the  $P_o$  species that we intended on measuring. Concentrations of  $P_e$  were estimated as the difference in measured  $P_i$  between sequential fractions with and without enzyme addition. The concentration of  $P_o$  that was resistant to enzymatic hydrolysis ( $P_{ne}$ ) for each extract was estimated as  $P_{ne} = P_t - (P_i + P_e)$ .

### Statistical Analyses

Pots were blocked by replication in the greenhouse; therefore, the study is best described as a randomized complete block design. Multiple regression analysis was used to evaluate the effects of rate on the  $P_t$  and  $P_i$ ; while ANOVA was used to evaluate the effects of rate on  $P_e$  and  $P_{ne}$  because the results showed

that there was no linearity between  $P_e$  and  $P_{ne}$  and rate. For the multiple regression, linear and nonlinear models were tested and the best model fit was selected using the Akaike Information Criteria (AIC) as the selection tool. In all cases, multiple regression or ANOVA, repeated measures analysis was used to separate out the errors associated with making several measurements on the same subject (soil sample) using Proc Glimmix in SAS 9.3 (SAS Institute, 2011), where each sequential fraction was regarded as the repeated variables. The variable P source was considered fixed effect; the variable rate was considered a continuous variable for  $P_t$  and  $P_i$  and a categorical variable for  $P_e$  and  $P_{ne}$ ; the replicates were considered a random effect (Littell et al., 2006). The AIC value was used as the model selection criteria to determine the best covariance model for the repeated variables, which in this case was the heterogeneous compound symmetry (CSH) for all model fits. Significant differences among treatments ( $P \leq 0.05$ ) were determined by mean separation using the multiple-comparison method as described by Westfall (2002). The multiple comparison procedure in SAS Proc GLIMMIX can control the overall Type I error rate within a mixed-model framework. All data were presented as the average of four replicates. This manuscript is mostly interested in evaluating the effects of biochar on soil P pools; therefore, significant interactions that do not involve P source (biochar addition) will not be discussed as in most cases those differences were due to the inherent physico-chemical and biological differences of the soils selected.

## RESULTS

### Phosphorus Concentration in each Sequential Fraction

Total P in each sequential fraction was affected by P source and application rate and soil type, which led to a soil by P source by rate by sequential fraction interaction (Table 2). Overall, the  $P_t$  in each sequential fraction and for each soil increased linearly as the amount of applied P increased (Table 2). The only exception was the water fraction, where no increase in  $P_t$  was observed for any soil (Table 2). The intercept in the equations represents the control treatment, which is the same for all P sources. Therefore, no discussion will be made for this parameter as no significant differences were observed for the intercepts for  $P_t$  (data not shown). For the Barnes soil,  $P_t$  extracted in  $\text{NaHCO}_3$  was found to have greater rate of increase for each unit of applied P when TSP was the P source, while the rate of increase was similar with the TMA, CCGb, and SPb (Table 2). The rate of increase in  $\text{NaOH}$ -extractable  $P_t$  for each unit of P applied was greater with TMA and TSP compared with CCGb and SPb (Table 2). No significant differences were observed in the rate of increase in  $\text{HCl}$ -extractable  $P_t$  for the Barnes soil (Table 2). For the Canisteo soil, the rate of increase in  $\text{NaHCO}_3$ -extracted  $P_t$  for each unit of applied P was greatest with CCGb and TSP and lowest with TMA and SPb (Table 2). In contrast, the rate of increase in  $\text{NaOH}$ -extractable  $P_t$  was lowest with CCGb compared with the other three P sources (Table 2). As observed for the Barnes soil, no significant changes in the rate of increase in  $\text{HCl}$ -extractable  $P_t$  was observed for the Canisteo soil (Table 2). For the Hubbard soil, the rate of increase in  $\text{NaHCO}_3$ -extractable  $P_t$  per unit of applied P was greater with TMA and CCGb, intermediate with TSP, and lowest with SPb (Table 2). The rate

Table 2. Estimated regression parameters for the increase in total P ( $P_t$ ) extracted by the different extractants used in the sequential fractionation.

Sequential fraction	P source†	Barnes			Canisteo			Hubbard		
		Intercept	+	Slope‡	Intercept	+	Slope	Intercept	+	Slope
		mg $P_t$ kg <sup>-1</sup>								
Water	Ash	2.22	+	0.007a¶	0.82	+	0.019a	1.96	+	0.022a
Water	CCGB	2.86	+	0.014a	0.79	+	0.020a	1.81	+	0.028a
Water	SPB	2.65	+	0.009a	0.87	+	0.019a	1.99	+	0.021a
Water	TSP	2.22	+	0.038a	0.44	+	0.027a	1.93	+	0.019a
NaHCO <sub>3</sub>	Ash	13.56	+	0.104b	28.77	+	0.031b	21.05	+	0.102a
NaHCO <sub>3</sub>	CCGB	13.80	+	0.116b	28.27	+	0.074a	21.43	+	0.100a
NaHCO <sub>3</sub>	SPB	12.82	+	0.121b	29.40	+	0.038b	22.08	+	0.068b
NaHCO <sub>3</sub>	TSP	15.17	+	0.156a	28.62	+	0.058a	21.89	+	0.088ab
NaOH	Ash	82.23	+	0.236a	121.57	+	0.247a	78.73	+	0.324a
NaOH	CCGB	81.87	+	0.130b	117.80	+	0.178b	72.30	+	0.193b
NaOH	SPB	86.93	+	0.159b	123.16	+	0.265a	78.72	+	0.308a
NaOH	TSP	80.95	+	0.222a	125.93	+	0.275a	78.34	+	0.310a
HCl	Ash	129.39	+	0.180a	58.27	+	0.077a	76.72	+	0.120b
HCl	CCGB	127.93	+	0.167a	57.00	+	0.082a	74.85	+	0.182a
HCl	SPB	126.96	+	0.167a	55.09	+	0.086a	75.52	+	0.136ab
HCl	TSP	127.80	+	0.177a	52.45	+	0.055a	74.93	+	0.147ab

† CCGB, corn cob gasification biochar; SPB, switchgrass pyrolysis biochar; TSP, triple superphosphate.

‡ LSD for the slope parameter for the water fraction 0.004 mg kg<sup>-1</sup>; NaHCO<sub>3</sub> fraction 0.019 mg kg<sup>-1</sup>; NaOH fraction 0.052 mg kg<sup>-1</sup>; and HCl fraction 0.050 mg kg<sup>-1</sup>.

¶ Means followed by the same letter for each soil and each sequential fraction are not significantly different ( $P \leq 0.05$ ).

of increase in NaOH-extractable  $P_t$  was lowest with CCGB than with the other three sources, as it was observed for the Canisteo soil (Table 2). Significant differences in how the P sources affected the HCl-extractable  $P_t$  were observed for the Hubbard soil. It was observed that CCGB had the greater rate of increase in HCl-extractable  $P_t$  per unit of P applied, with TSP and SPB intermediate, and TMA the lowest (Table 2).

Inorganic P in each sequential fraction was affected by P source, application rate, and soil type, which led to a soil by P source by rate by sequential fraction interaction (Table 3). Overall, the  $P_i$  in each sequential fraction and for each soil increased linearly as the amount of applied P increased (Table 3). The only exception was the water fraction, where no increase in  $P_i$  was observed for any soil (Table 3). In addition, no significant differences were observed for the intercept parameter among the different P sources within each soil; therefore, no discussion will be done for this parameter in this fraction. The rate of increase in NaHCO<sub>3</sub>-extractable  $P_i$  in the Barnes soil for each unit of P applied was greatest for the CCGB source, followed by TMA, and lowest with SPB and TSP (Table 3). No differences were observed in the rate of increase in NaOH-extractable  $P_i$  in the Barnes soil (Table 3). The HCl-extractable fraction was affected the most with TMA and the least with the other three sources (Table 3). In the Canisteo soil, NaHCO<sub>3</sub>-extractable  $P_i$  increased the most when CCGB and TSP was used compared with TMA and SPB (Table 3). The NaOH-extractable  $P_i$  had the greatest rate of increase per P applied when TMA was the P source, while the results for the other three sources were similar (Table 3). Inorganic P extractable in HCl was increased the most with SPB, intermediate with TSP, and the least with the TMA and CCGB (Table 3). In the Hubbard soil, the greatest rate of increase in NaHCO<sub>3</sub>-extractable  $P_i$  per unit of P applied was observed for CCGB, followed by TSP, and lowest with TMA and SPB (Table 3). Inorganic P extracted in NaOH

increased the most with TMA, while no differences were observed among the other three sources (Table 3). No significant differences were observed in the rate of increase in soil  $P_i$  for the HCl-extractable  $P_i$  in the Hubbard soil (Table 3).

### Non-hydrolysable and Hydrolysable P

As mentioned earlier, the results for the  $P_e$  and  $P_{ne}$  were found to be inconsistent and in most cases they did not fit a linear or quadratic model; therefore, these data sets were analyzed with ANOVA as opposed to regression analysis. There were cases where linear responses were observed, but we opted for analyzing the data using ANOVA. Nonetheless, the results presented are clear, and the reader can make their own assumptions where a linear increase is clearly visible in the results; furthermore, the mean separation can also be used to see the increases. The interactions between P source by rate by sequential fraction as well as soil by rate by sequential fraction were found to be significant ( $P \geq 0.05$ ) for  $P_{ne}$ . There was a significant increase in water-extractable  $P_{ne}$  after the addition of TSP and CCGB, a slight upward trend with the addition of TMA, and no significant changes after the addition of SPB (Table 4). The NaHCO<sub>3</sub>- and NaOH-extractable  $P_{ne}$  increased as the P application rate increased (Table 4). In contrast to the results observed for the first three sequential fractions, the HCl-extractable  $P_{ne}$  decreased after P application (Table 4). In addition, TSP application lead to lower extractable  $P_{ne}$  than TMA, CCGB, and SPB especially as the application rate increased (Table 4).

For the extractable  $P_e$ , it was observed that the interactions soil by P source by rate and also soil by rate by sequential fraction were significant and therefore will be discussed. For the Barnes soil, application of TMA and CCGB tended to decrease the amount of extractable  $P_e$ , while the application of SPB and TSP increased extractable  $P_e$  in comparison with the untreated control (Table 5). For the Canisteo soil, application of TMA

Table 3. Estimated regression parameters for the increase in inorganic P ( $P_i$ ) extracted by the different extractants used in the sequential fractionation.

Sequential fraction	P source†	Barnes			Canisteo			Hubbard		
		Intercept		Slope‡	Intercept		Slope	Intercept		Slope
		mg P <sub>i</sub> kg <sup>-1</sup>								
Water	Ash	0.059	+	0.008a¶	-0.092	+	0.004a	-0.128	+	0.006a
Water	CCGB	-0.013	+	0.013a	-0.123	+	0.006a	-0.163	+	0.009a
Water	SPB	0.020	+	0.011a	-0.096	+	0.004a	-0.108	+	0.004a
Water	TSP	0.049	+	0.022a	-0.173	+	0.007a	-0.154	+	0.006a
NaHCO <sub>3</sub>	Ash	7.511	+	0.034ab	5.327	+	0.056b	3.103	+	0.022c
NaHCO <sub>3</sub>	CCGB	6.814	+	0.041a	4.713	+	0.067a	3.098	+	0.064a
NaHCO <sub>3</sub>	SPB	7.038	+	0.032b	4.684	+	0.053b	2.962	+	0.030c
NaHCO <sub>3</sub>	TSP	7.508	+	0.029b	4.485	+	0.076a	3.393	+	0.042b
NaOH	Ash	21.237	+	0.094a	28.505	+	0.095a	15.081	+	0.163a
NaOH	CCGB	22.066	+	0.092a	28.165	+	0.047b	14.432	+	0.069b
NaOH	SPB	22.598	+	0.076a	28.363	+	0.057b	14.633	+	0.088b
NaOH	TSP	21.701	+	0.106a	28.303	+	0.064b	14.524	+	0.095b
HCl	Ash	54.720	+	0.325a	44.346	+	0.086b	14.750	+	0.077a
HCl	CCGB	53.929	+	0.263b	43.124	+	0.082b	12.996	+	0.096a
HCl	SPB	50.314	+	0.240b	40.320	+	0.131a	13.582	+	0.071a
HCl	TSP	53.431	+	0.223b	40.338	+	0.100ab	11.900	+	0.088a

† CCGB, corn cob gasification biochar; SPB, switchgrass pyrolysis biochar; TSP, triple superphosphate.

‡ LSD for water fraction 0.002 mg kg<sup>-1</sup>; NaHCO<sub>3</sub> fraction 0.009 mg kg<sup>-1</sup>; NaOH fraction 0.029 mg kg<sup>-1</sup>; and HCl fraction 0.044 mg kg<sup>-1</sup>.

¶ Means followed by the same letter for each soil and each sequential fraction are not significantly different ( $P \leq 0.05$ ).

Table 4. Soil non-hydrolyzable organic P ( $P_{ne}$ ) averaged across soils as affected by increasing biochar application rates in comparison with triple superphosphate (TSP) in three soils collected from agricultural fields in west central, central, and southwest Minnesota.

Sequential fraction	Rate	TMA†	CCGB	SPB	TSP
		mg P kg <sup>-1</sup>	$P_{ne}$ , mg P kg <sup>-1</sup>		
Water	0	0.33ab‡	0.33c	0.33a	0.33c
Water	28	0.14b	0.48bc	0.51a	0.46c
Water	56	0.36ab	0.76ab	0.30a	0.98b
Water	84	0.59a	1.04a	0.58a	2.08a
NaHCO <sub>3</sub>	0	12.31d	12.31d	12.31c	12.31d
NaHCO <sub>3</sub>	28	15.59c	15.81c	16.88b	18.59c
NaHCO <sub>3</sub>	56	17.88b	18.29b	17.59ab	20.52b
NaHCO <sub>3</sub>	84	21.29a	21.78a	20.29a	22.41a
NaOH	0	59.02d	59.02c	59.02d	59.02d
NaOH	28	69.45c	60.68c	75.83c	73.88c
NaOH	56	82.63b	70.22b	82.25b	85.22b
NaOH	84	93.08a	83.09a	95.12a	94.18a
HCl	0	38.96a	38.96a	38.96a	38.96a
HCl	28	28.26c	29.01bc	20.74b	19.11b
HCl	56	32.88b	32.55b	20.68b	18.09b
HCl	84	27.53c	26.56c	23.92b	16.34b

† TMA, turkey manure ash; CCGB, corn cob gasified biochar; SPB, switchgrass pyrolysis biochar.

‡ Means followed by different letter within a sequential fraction for each P source are significantly different ( $P < 0.05$ ). Protected least significant differences for the sequential fractions water, NaHCO<sub>3</sub>, NaOH, and HCl were 0.36, 1.69, 3.66, and 4.83, respectively.

and CCGB also decreased extractable  $P_e$ , while the application of SPB increased  $P_e$  only at the rate of 28 kg  $P_2O_5$  ha<sup>-1</sup>, the other rates caused no changes in extractable  $P_e$ , and no changes were observed after TSP application compared with the control (Table 5). For the Hubbard soil, application of TMA, SPB, and TSP had no effects on extractable  $P_e$ , while CCGB at the rate

Table 5. Soil hydrolyzable organic P ( $P_e$  = monoester-P + DNA-P + phytate P) averaged across soils as affected by biochar application in comparison with triple superphosphate (TSP) in three soils collected from agricultural fields in west central, central, and southwest Minnesota.

P source†	Rate	Barnes	Canisteo	Hubbard
		mg P kg <sup>-1</sup>	$P_e$ , mg P kg <sup>-1</sup>	
TMA	0	4.95a‡	6.59a	12.13a
TMA	28	6.11a	6.82a	13.48a
TMA	56	3.77ab	3.88b	11.14a
TMA	84	1.94b	3.22b	11.89a
CCGB	0	4.95b	6.59a	12.13a
CCGB	28	8.52a	6.94a	12.03a
CCGB	56	4.80b	4.96b	9.86b
CCGB	84	7.03ab	3.14b	12.82a
SPB	0	4.95c	6.59b	12.13ab
SPB	28	13.82a	9.40a	13.29a
SPB	56	11.82a	6.87b	10.13b
SPB	84	8.73b	4.74b	13.08a
TSP	0	4.95b	6.59a	12.13a
TSP	28	14.44a	6.78a	11.95a
TSP	56	15.59a	4.94a	10.85a
TSP	84	16.23a	4.56a	10.50a

† TMA, turkey manure ash; CCGB, corn cob gasified biochar; SPB, switchgrass pyrolysis biochar.

‡ Means followed by different letter within the column for each P source are significantly different ( $P < 0.05$ ). Protected least significant differences for the rates 0, 28, 56, and 84 mg P kg<sup>-1</sup> were 2.37, 2.45, 2.45, and 2.45, respectively.

of 28 kg  $P_2O_5$  ha<sup>-1</sup> caused a decreased in extractable  $P_e$  compared with the untreated control (Table 5).

Among the three  $P_e$  forms studied in this research, the monoester-like  $P_e$  was found to be affected the most by biochar addition. The application TMA in most cases caused an initial increase in extractable monoester-like P in all sequential fractions with a decrease in extractable monoester-like P as the

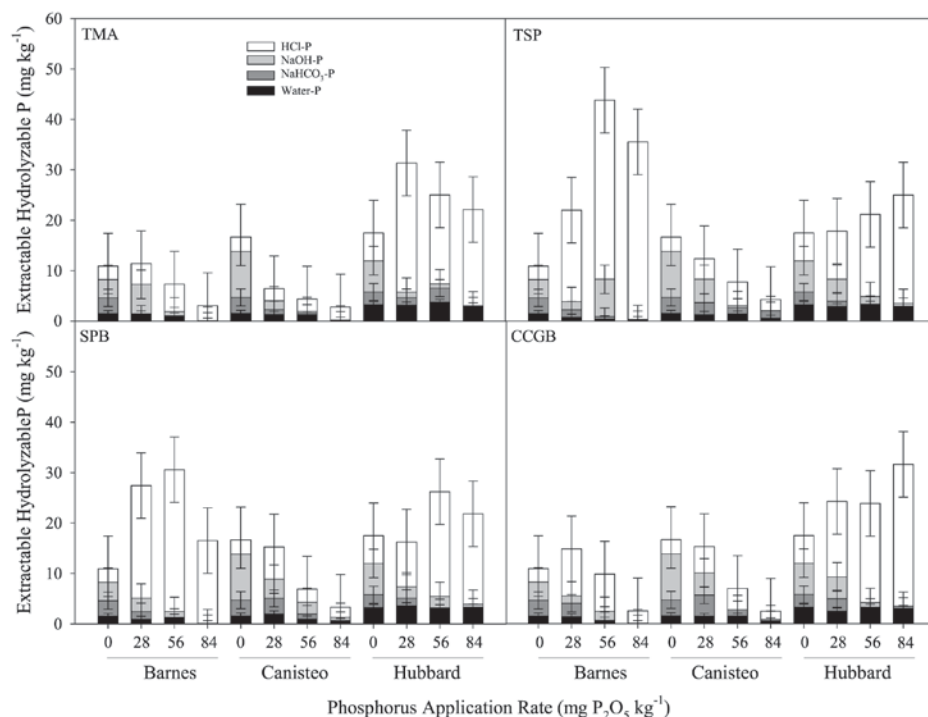


Fig. 1. Monoester-like P extracted in each fraction as affected by increasing biochar application rates and triple superphosphate (TSP) in three soils collected from agricultural fields in west central, central, and southwest Minnesota. Error bars represent the least significant difference.

application rate increased (Fig. 1). The only exception was the HCl fraction in the Hubbard soil where levels increased with the first application rate and stayed high with the higher application rates (Fig. 1). Application of CCGB caused an overall decrease in monoester-like P in the water,  $\text{NaHCO}_3$ , and NaOH fractions of all three soils. However, CCGB application caused an initial increase in HCl-extractable  $P_e$  with the first rate and a decrease with the intermediate and highest rates in the Barnes and Canisteo soils, and a steady increase with each rate in the Hubbard soil (Fig. 1). Application of SPB and TSP led to an overall decrease in monoester-like P in the water,  $\text{NaHCO}_3$ , and NaOH fractions in soils (Fig. 1). In addition, SPB and TSP also tended to increase monoester-like P in the soils (Fig. 1). For the DNA-like P, biochar addition caused a significant soil by P source interaction; this being the only significant interaction involving P source it is the only one discussed for this P form. In general, CCGB and SPB were found to increase DNA-like P more than the TMA in the NaOH and HCl fractions (Table 6). Corn-cob biochar also was found to increase the amount of DNA-like P in the  $\text{NaHCO}_3$  compared with the other three sources (Table 6). Phytate-like  $P_e$  was not affected by any P source used in this study.

## DISCUSSION

Biochars were found to be less effective at increasing extractable  $P_e$  compared with the TSP and this effect was more evident in the Barnes soil than in the Canisteo and Hubbard soil. It is possible that this was due to the higher pH observed for the Barnes soil compared with the other two soils (Table 1). The effectiveness of TMA at increasing extractable  $P_e$  in most cases was similar to TSP. Most of the biochar effects on soil extractable  $P_e$  were observed for the  $\text{NaHCO}_3$  and NaOH sequential fractions, which are considered labile and sparingly soluble fractions (Hedley et al., 1982). These two extractants

Table 6. Soil hydrolyzable organic P ( $P_e$ ) averaged across soils as affected by biochar application in comparison with triple superphosphate (TSP) in three soils collected from agricultural fields in west central, central, and southwest Minnesota.

Sequential fraction	TMA†	CCGB	SPB	TSP
	DNA-like $P_e$ , mg P kg <sup>-1</sup>			
Water	0.36 a‡	0.34 a	0.35a	0.34a
$\text{NaHCO}_3$	0.23 b	0.05 b	0.65a	0.16b
NaOH	4.63 b	5.28ab	5.75a	5.27ab
HCl	3.14b	5.31 a	6.67a	6.71a

† TMA, turkey manure ash; CCGB, corn cob gasified biochar; SPB, switchgrass pyrolysis biochar.

‡ Means within a row followed by different letters are significantly different ( $P < 0.05$ ). Protected least significant differences for the sequential fractions water,  $\text{NaHCO}_3$ , NaOH, and HCl were 0.12, 0.32, 0.97, and 1.92, respectively.

are known for removing P bound to specific sites in the soil;  $\text{NaHCO}_3$  removes loosely Ca-bound P, and the NaOH removes Al and Fe bound P. The fact that CCGB and SPB increased the  $\text{NaHCO}_3$  and NaOH the least compared with TMA and TSP suggests that a portion of P from those sources gets occluded (bound to insoluble compounds) or sorbed to sites that are not accessible to these extractants (Lehmann, 2007b; Hale et al., 2013; Satriawan and Handayanto 2015).

The CCGB was found to have the highest effectiveness at increasing the  $\text{NaHCO}_3$  extractable  $P_e$  compared with the three sources, and the SPB was found to affect this sequential fraction similarly to TSP and TMA. The  $\text{NaHCO}_3$  reflects the soil P pool that most likely reflects the soil P fraction that is plant available. Liang et al. (2014) reported that P release from a dairy manure biochar was initially controlled by diffusion, followed by slow and steady dissolution of whitlockite, a Ca and Mg phosphate mineral formed during biochar production. Hale et al. (2013) reported that biochar from corn cob provided a slow release of phosphate to soils. Ch'ng et al. (2014)



saw that the addition of chicken litter biochar to an acidic soil increased soil available P as well as Al and Fe bound P (Ch'ng et al., 2014). Pagliari et al. (2009) found that TMA application resulted in alfalfa biomass yield equivalent to TSP-treated plots in a field study conducted for 2 yr. In a separate study, Pagliari et al. (2010a) reported similar P availability from TMA and TSP as corn P uptake following application of TMA was equivalent to corn grown in TSP-treated soil. Therefore, based on the results observed for  $P_i$  in the present study it is likely that the biochars tested could be as effective as commercial fertilizer at providing plants with adequate levels of  $P_i$ .

Soil  $P_o$  is a significant source of  $P_i$  in soils. Understanding how biochar affects this pool can help in the development of sound scientific best management practices. Because the  $P_o$  fraction is actually made up of two distinct fractions, one that can be mineralized and one that cannot, one should investigate both and see if biochar has any effect on those pools. In general, no consistent results were observed for the effects of biochar on the soil  $P_e$ , and, in fact, the results were contradictory and in most cases random. For example, in the Barnes soil, CCGB application increased extractable  $P_e$  only in the lowest rate, with a trend of an increase in the highest rate, while in the Hubbard soil, application of the 56 kg  $P_2O_5$  ha<sup>-1</sup> decreased soil  $P_e$  compared with the control (Table 5). The application of SPB in the Canisteo at the lowest rate increased soil  $P_e$  compared with the control while the higher application rates had the same extractable  $P_e$  as the control (Table 5). In contrast, the effects of TMA and TSP on soil extractable  $P_e$  were more consistent; TMA tended to decrease soil  $P_e$  while TSP either increased compared with the control (Barnes soil) or kept the levels similar to those in the control (other soils) (Table 5). The fact that each biochar source had a different behavior based on the soil it was applied to shows that a generalization of the effects of a given biochar to soils with different properties should be avoided. Furthermore, the results of this research suggest that field research should be conducted to evaluate the long-term effects of biochar on the different soil P pools in soils with different properties

## CONCLUSION

The application of biochar led to significant changes in the distribution of total P in soils receiving biochar compared with soils receiving TSP. In addition, the rate of increase per unit of P applied was lower for soils receiving biochar compared with soils receiving TSP. Although there were significant changes in the total P pools, the inorganic P was less affected by biochar addition. In most cases, the amount of inorganic P assumed to be labile, or bioavailable, was similar between biochar and TSP. The commercial fertilizer solubilized higher levels of HCl-soluble non-hydrolysable P than the biochars, suggesting that biochar has lower effects on the Ca-bound P than commercial fertilizer. Inconsistent trends were observed for the enzymatically hydrolysable P. In most cases, biochar either decreased or did not change hydrolysable P compared with the untreated soil or soil treated with TSP. Overall, the results of this showed that biochar can significantly change the distribution of P in soils; however, the changes will depend on the biochar and soil properties.

## ACKNOWLEDGMENTS

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